

## REVIEW

# Hepatitis E: a complex and global disease

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Thirty years after its discovery, the hepatitis E virus (HEV) continues to represent a major public health problem in developing countries. In developed countries, it has emerged as a significant cause of non-travel-associated acute hepatitis. HEV infects a wide range of mammalian species and a key reservoir worldwide appears to be swine. Genomic sequence similarity between some human HEV genotypes and swine HEV strains has been identified and we know that humans can acquire HEV infection from animals. Although for the most part the clinical course of HEV infection is asymptomatic or mild, significant risk of serious disease exists in pregnant women and those with chronic liver disease. In addition, there are data on the threat of chronic infections in immunocompromised patients. Beyond management of exposure by public health measures, recent data support that active immunisation can prevent hepatitis E, highlighting the need for vaccination programmes. Here we review the current knowledge on HEV, its epidemiology, and the management and prevention of human disease.

## Introduction

First recognised in Asia almost 30 years ago as the main cause of non-A, non-B enterically transmitted hepatitis,<sup>1,2</sup> hepatitis E virus (HEV) is now acknowledged to have worldwide distribution.

In countries with poor sanitation, HEV is endemic and typically causes explosive outbreaks of acute hepatitis, usually associated with faecal contamination of the water supply. The disease is generally mild, yet pregnant women suffer significant morbidity and mortality.<sup>3-5</sup>

In contrast, in countries with high standards of sanitation, hepatitis E occurs sporadically, initially identified as an imported disease in travellers from highly endemic regions, but subsequently diagnosed in patients with no travel history as well; this latter form has been named 'hepatitis E indigenous to developed countries'.<sup>6-26</sup>

Phylogenetic analysis of HEV genome from different isolates has led to the identification of four main genotypes, with genotypes 1 and 2 circulating in Africa and Asia, genotype 3 showing a broad distribution worldwide, and genotype 4 being restricted to Asia.

Genotypes 3 and 4 are enzootic in a variety of wild and domestic animals, particularly pigs,<sup>8,27-36</sup> which gave rise to the question of whether human HEV infection is a zoonosis? Evidence from Japan<sup>37-40</sup> and China<sup>28</sup> now confirms that humans can acquire HEV infection from animals.

Hepatitis E represents a significant public health and economic burden particularly in countries where the absence of sanitation infrastructures, or their breakdown as a consequence of wars or natural disasters, brings the hygienic conditions below a safe level.<sup>4,41,42</sup> The development of an effective vaccine is expected to dramatically reduce the incidence of the disease, particularly in the most susceptible individuals such as pregnant women.

## Virology

### Taxonomy and virus structure

In the early 1980s, the observation that individuals involved in epidemics of jaundice were seronegative for markers of acute hepatitis A and B suggested the existence of an unrecognised aetiological agent of enterically transmitted hepatitis.<sup>1,2</sup> The confirmation came in 1983, when small, virus-like particles were identified by immune electron microscopy in stool specimens from a volunteer experimentally infected with pooled faecal extracts from human cases of epidemic non-A, non-B hepatitis.<sup>43</sup> The pathogen, HEV, is a non-enveloped virus, 27–30 nm in diameter, with an icosahedral capsid. In the early 1990s, the virus genome was cloned<sup>44,45</sup> and diagnostic antibody assays were developed.<sup>46</sup> After being provisionally assigned to the *Caliciviridae* family,<sup>47</sup> HEV was re-classified as the sole member of the genus *Hepevirus*, family *Hepeviridae*, in 2004.<sup>48</sup>



### Genomic organisation and viral proteins

The HEV genome consists of a single-stranded RNA molecule with positive polarity approximately 7300 nucleotides in length.<sup>45,49,50</sup> It comprises a short 5' noncoding region (28 nucleotides), followed by three open reading frames (ORF), a 3' noncoding region (65–74 nucleotides), and a poly(A) tail (Figure 1).<sup>45,53</sup> The genome is capped at its 5' terminus<sup>54</sup> and capping is required for virus viability.<sup>55</sup>

ORF1 (approximately 5 kb) encodes a large nonstructural polyprotein with key functions for viral genome replication and viral protein processing. ORF2 (approximately 2 kb) occupies the 3' end of the coding region and encodes the capsid protein.<sup>45,51,56–58</sup> The N-terminal region of ORF2 protein binds the 5' noncoding region of the HEV genome<sup>59</sup> and is possibly involved in viral encapsidation.<sup>41</sup> Only the ORF2 recombinant protein truncated at its N-termini can efficiently self-assemble *in vitro* into empty, virus-like particles. These share antigenic properties with the native HEV capsid protein, although they are smaller than the native virions.<sup>57,58</sup>

ORF3 is a small reading frame (372 bases) with the 5' end overlapping ORF1 by four nucleotides and the 3' end overlapping ORF2 by 331 nucleotides;<sup>45,53,51</sup> it encodes a small phosphoprotein that associates with the cytoskeleton and the capsid protein.<sup>60,61</sup> The product of ORF3 is possibly involved in modulation of cell signalling<sup>62,63</sup> and in the assembly of the HEV nucleocapsid;<sup>60</sup> recently, it has been shown that this protein is essential for infectivity *in vivo*<sup>64</sup> but not *in vitro*.<sup>65</sup> The poly(A) tail is necessary for binding of RNA-dependent RNA polymerase (RdRp) to the 3' noncoding region.<sup>41,66</sup>

Compared with genotypes 1–3, the genome of genotype 4 contains a nucleotide insertion (U) just after the second AUG codon of ORF3, which changes the downstream reading frames so that, for genotype 4, different AUG codons were initially expected to initiate translation in both ORF2 and ORF3.<sup>52,53</sup> However, a recent study by Graff *et al.*,<sup>51</sup> using a replicon, demonstrated that ORF2 and ORF3 proteins are produced from a single subgenomic RNA of approximately

2.2 kb, which initiates downstream of the first two AUG codons of ORF3 in genotypes 1–3 (downstream of the insertion of U in genotype 4), using two closely spaced start codons. It is therefore expected that both ORF2 and ORF3 proteins are similar in size in all four genotypes.<sup>51</sup>

### Resistance to physical and chemical agents

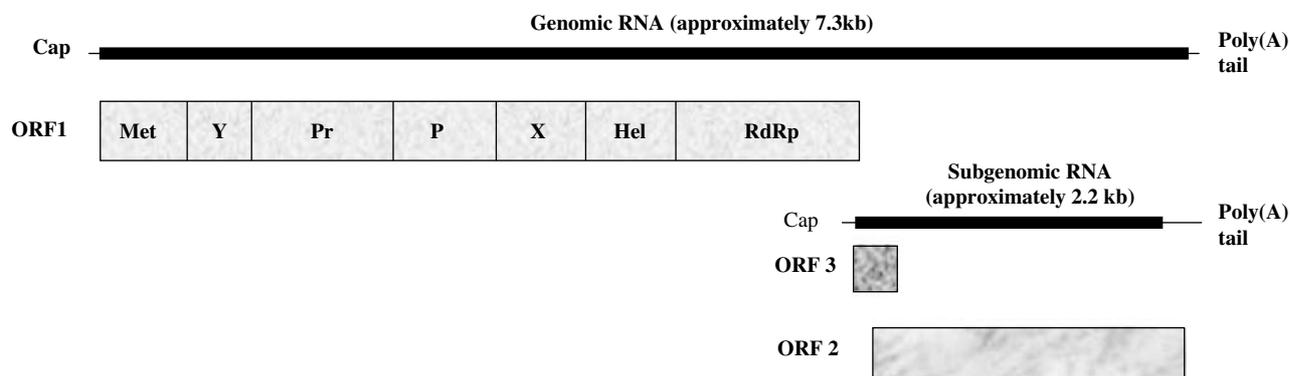
Boiling and chlorination of water represent the main measures to control and prevent infection. However, Emerson *et al.*<sup>67</sup> reported that HEV is moderately resistant to heat inactivation. This finding was further corroborated in a more recent study that showed how HEV was still infectious on incubation at 56°C for 30 min.<sup>68</sup>

### HEV genetic diversity

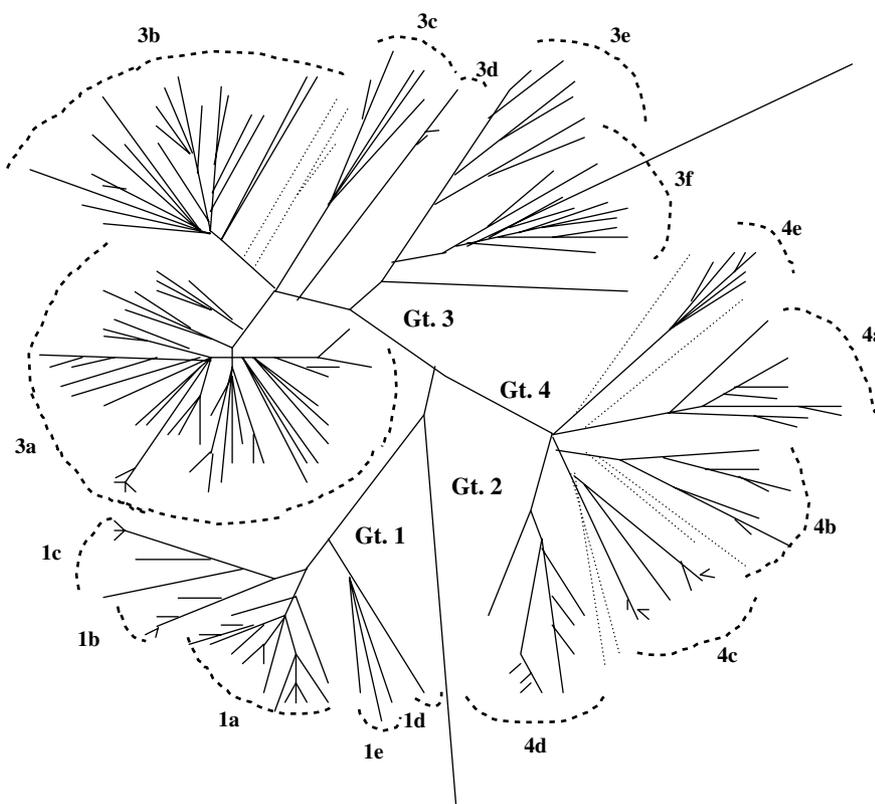
Comparative nucleotide sequence analysis of whole genomes of HEV isolates has revealed extensive genomic diversity leading to the identification of four major genotypes and several subtypes within each genotype (Figure 2). However, although the separation of HEV into four major genogroups is widely accepted, so far there is no agreement about the number of subtypes within each genotype.<sup>69</sup> Genomic regions that have been used for phylogenetic purposes include a 301-nucleotide-long sequence at the 5' end of the ORF2 region (Figure 2)<sup>69</sup> and a 306-nucleotide-long sequence in the RdRp of ORF1.<sup>70</sup>

### Replication in cell culture

The lack of efficient cell culture systems has hampered detailed studies on HEV biology, critical for helping to develop diagnostic assays and vaccine research.<sup>68</sup> Replication and propagation of HEV was attempted with limited success by using continuous cell lines<sup>71,72</sup> and primary hepatocytes from nonhuman primates.<sup>73</sup> Recently, more efficient cell-culture systems were developed in cell lines,<sup>67,68,74</sup> allowing studies on HEV thermal stability and improving neutralisation tests. It was shown that anti-HEV antibodies are broadly crossreactive because HEV genotype 3 was neutralised by convalescent serum samples from patients



**Figure 1** Schematic organisation of genomic and subgenomic HEV RNAs. ORF1 encodes a nonstructural polyprotein; ORF2 encodes the capsid protein; ORF3 encodes a phosphoprotein. Met, methyltransferase; Y, no function assigned at present; Pr, putative papain-like cysteine protease; P, proline 'hinge'; X, no function assigned at present; Hel, RNA helicase; RdRp, RNA-dependent RNA polymerase.<sup>51,52</sup>



**Figure 2** Phylogeny of HEV genotypes (Gt.) based on the 301-nucleotide-long 5' end of the ORF2 region, the most conserved in all HEV isolates. Sequences determined from this region accounted for the majority of HEV sequences.<sup>69</sup>

infected with HEV genotypes 1, 3, and 4.<sup>68</sup> Similarly, genotype 1 was neutralised by convalescent serum samples obtained from rhesus monkey infected with any of the four mammalian genotypes.<sup>74</sup> In addition, serum specimens obtained 24 years after the onset of HEV infection could prevent the propagation of HEV in cell culture, suggesting that long-lasting HEV antibodies with neutralisation activity are induced.<sup>68</sup>

## Epidemiology

### Geographical distribution of HEV according to genotype

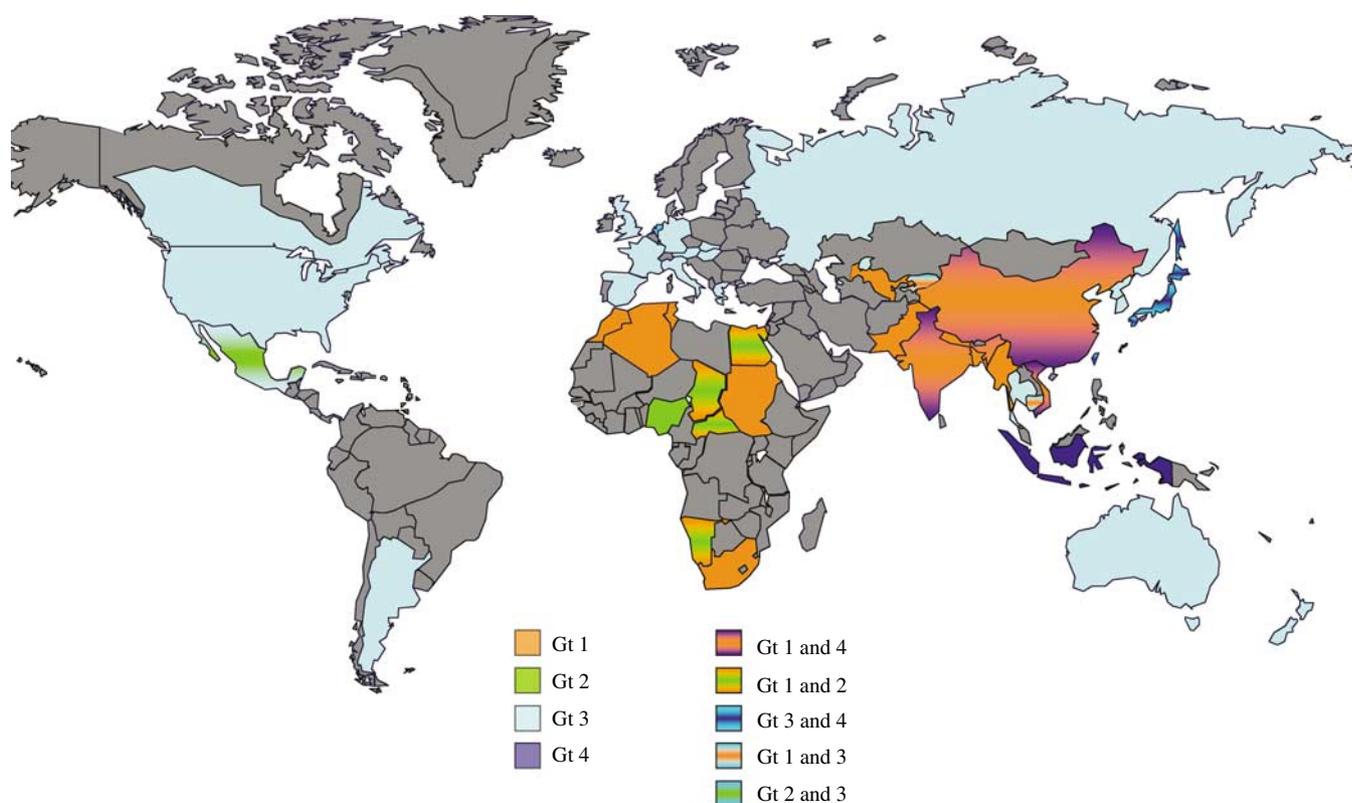
The geographical distribution of HEV genotypes is complex and continuously evolving (Figure 3). Genotype 1 extensively circulates in Asia (including India,<sup>75–78</sup> Pakistan,<sup>79</sup> Nepal,<sup>80,81</sup> Bangladesh,<sup>82</sup> China,<sup>49,83–85</sup> Kyrgyzstan,<sup>86</sup> and Uzbekistan<sup>87</sup>) and Africa (including Egypt,<sup>88</sup> Algeria,<sup>89</sup> Morocco,<sup>87</sup> Namibia,<sup>90</sup> Sudan,<sup>91</sup> and Chad<sup>91,92</sup>), whereas genotype 2 has been isolated only in Mexico<sup>93</sup> and in some African countries (Nigeria,<sup>94</sup> Namibia,<sup>95</sup> Chad,<sup>91</sup> and Sudan<sup>91</sup>). Genotype 3 has been detected worldwide (America,<sup>13,26,32,33,96–99</sup> Europe,<sup>9,10,12,15,16,18,20,21,27,34,35,100–102</sup> Asia,<sup>25,33,37,39,40,103–111</sup> Australia,<sup>69</sup> and New Zealand<sup>11</sup>) with the exception of Africa, whereas genotype 4 is restricted to India<sup>112,113</sup> and East Asia.<sup>39,84,103,114–118</sup>

Although genotypes 1 and 2 are considered human viruses, genotypes 3 and 4 have been isolated from both humans<sup>10,11,16,17,20,25,97,119–122</sup> and animals.<sup>32,33,123–126</sup> Of significant interest is the unique distribution of HEV isolates in India, as human and swine HEVs belong to different genotypes (genotype 1 and genotype 4, respectively);<sup>113</sup> genotype 4 appears to be specifically restricted to swine and it has never been isolated from humans, in spite of extensive investigations.<sup>127,128</sup> Phylogenetic analysis shows that the Indian HEV genotype 4 represents a distinct variant among HEV genotype 4 isolates with 26 unique amino-acid substitutions (16 in ORF1, 8 in ORF2, and 2 in ORF3).<sup>129</sup> Whether the difference in sequence, particularly in ORF2, determines tropism and explains the lack of infectivity in humans remains to be determined.

### Mode of transmission

#### Waterborne

In developing countries, HEV is transmitted through the faecal–oral route, mainly by the consumption of water contaminated with sewage disposal.<sup>130</sup> In developed countries, HEV RNA has been detected in human sewage only occasionally.<sup>19,20</sup> Interestingly, HEV rescued from sewage in Spain was infectious for rhesus monkey,<sup>131</sup> raising the possibility that HEV might occasionally



**Figure 3** Geographical distribution of HEV isolates according to genotypes (Gt). HEV Gt 1 and 2: epidemic strains causing human infection. HEV Gt 3 and 4: zoonotic strains isolated from humans and a variety of animals, particularly pigs. In some countries, different genotypes co-circulate in distinct ecological niches: Gt 1 and 4 in China, India, and Vietnam; Gt 1 and 2 in several African countries, including Namibia, Chad, and Sudan; Gt 3 and 4 in Japan; Gt 1 and 3 in Cambodia; Gt 2 and 3 in Mexico.

contaminate the environment and shellfish even in non-epidemic regions.<sup>16,131,132</sup>

#### Foodborne

Evidence that food can transmit HEV came from Japan, where acute hepatitis E was diagnosed in patients who consumed raw or undercooked pig liver and intestine,<sup>23</sup> wild boar meat and liver,<sup>38,110,133</sup> and deer meat<sup>40</sup> contaminated with the virus. HEV with identical nucleotide sequence was detected both in the blood of affected patients and in batches of meat and liver not consumed.<sup>40,109,133</sup> A higher HEV IgG seroprevalence in Japanese individuals with frequent dietary consumption of raw deer meat, in comparison with a control group, indirectly supports the foodborne route.<sup>134</sup> Food as a vehicle of infection has not yet been proven in other developed countries.

#### Person-to-person transmission

In contrast to hepatitis A virus (HAV) infection, secondary transmission among household members of patients with acute hepatitis E is an uncommon event,<sup>135,136</sup> both in the context of outbreaks<sup>137</sup> and sporadic infections.<sup>138,139</sup>

#### Parenteral transmission

HEV-infected individuals can transmit the infection by donating blood during the viraemic period. Viraemia can be detected even in asymptomatic infections and during the incubation period,<sup>140,141</sup> even in the absence of aminotransferase elevation.<sup>140</sup> Transmission of HEV via blood transfusion has been documented in several countries, including Saudi Arabia,<sup>142</sup> Japan,<sup>105,143,144</sup> and the UK,<sup>145</sup> where matching RNA sequences were found in blood donors and their recipients.

#### Mother-to-child transmission

Mother-to-child transmission of HEV has been scarcely documented; however, the available data suggest a significant rate of HEV vertical transmission among the HEV RNA-positive mothers with worsening liver disease.<sup>146-149</sup>

#### Epidemic versus sporadic forms and seasonality

In the developing world, HEV infection represents the most common aetiological agent of periodic outbreaks of acute hepatitis.<sup>42,130,136,150</sup> Epidemics are most frequent during the monsoon season when flooding causes faecal contamination

of drinking water.<sup>41</sup> Between epidemics, HEV is transmitted in a discrete manner leading to the onset of sporadic forms of acute hepatitis. In India, 30–70% of all cases of acute sporadic hepatitis are caused by HEV infection.<sup>41,151</sup>

In economically developed regions, indigenous hepatitis E is largely a sporadic disease. However, in Japan, small outbreaks have been described as a consequence of consumption of the same contaminated food.<sup>152,153</sup> A small outbreak involving a family with two children has also been reported in France.<sup>139</sup> No clear seasonality has been observed in developed countries.<sup>24,154</sup>

#### Age- and sex-specific clinical attack rates and case fatality rates

Clinical infection concentrates among adolescents and young adults in countries of high endemicity. During outbreaks, the clinical attack rate (3–30%)<sup>136,150,155–157</sup> is highest among pregnant women.<sup>4,158,159</sup> The mortality rate, which is usually low (0.07–0.6%),<sup>136,150,160,161</sup> can reach values as high as 25–31% in pregnant women, particularly during the third trimester of pregnancy.<sup>4,136,150,155,162–164</sup>

A distinct characteristic of hepatitis E indigenous to developed countries is its high attack rate among older male adults.<sup>13,26,100</sup> This has been observed in both Europe<sup>10,16,18,20,100,101</sup> and Japan,<sup>24</sup> which experienced the greatest number of detected cases. No documented case of hepatitis E has been identified in pregnant women in this different epidemiological setting.

A high mortality, ranging between 25 and 70%, has been recently documented among patients already suffering from chronic liver disease of different aetiologies. These observations were made both in highly endemic countries, such as India,<sup>165–168</sup> Pakistan,<sup>169</sup> and Nepal,<sup>170</sup> where hepatitis E is caused by genotype 1, and in Europe,<sup>171</sup> where indigenous hepatitis E is linked to genotype 3.

#### Seroprevalence of HEV infection

HEV seroprevalence studies have been conducted using several antibody assays based on different recombinant antigens, which do not always include the most relevant B-cell epitopes, leading to lack of sensitivity and poor reciprocal concordance.<sup>172</sup> In particular, a greater sensitivity of currently available antibody assays has been reported in detecting overt disease in comparison with subclinical infections,<sup>173</sup> which may hamper seroepidemiological investigations. Antibody assays based on the ORF2 protein, which exposes at least one major crossreactive epitope shared by all HEV genotypes,<sup>56,62,174</sup> should be used for investigating seroepidemiology<sup>46,56,175,176</sup> (see 'Diagnosis').

Seroprevalence studies in HEV epidemic countries, such as India, have revealed that HEV infection is rare in children, reaching peak prevalence (33–40%) only in early adulthood.<sup>177</sup> These data are in striking contrast with HAV serosurveys, showing anti-HAV antibodies in the majority of children by the age of three years.<sup>177</sup> The reason for the

difference in age-specific seroprevalence between HAV and HEV, both transmitted by the faecal–oral route, remains unanswered.

In contrast, HEV seroepidemiology closely mirrors that of HAV in Egypt, a country highly endemic for HEV infection (similar to India), where anti-HEV antibodies are detectable in 65% of children younger than 10 years.<sup>178</sup> Although the reason for the earlier exposure in life in Egypt remains unknown, the widespread immunity of the population at an early age might account for the absence of large outbreaks of hepatitis E in the general population<sup>179</sup> and among pregnant women.<sup>180</sup>

In rural southern China, where the majority of HEV infections are zoonotic (genotype 4), anti-HEV IgG is rarely detected in children, rapidly increases in young adults, and peaks (60–80%) at the age of 60 years.<sup>181</sup> Interestingly, after 30 years of age, but not in younger age groups, the seroprevalence is two times higher for men than for women, suggesting a link with different social roles adopted by men and women once families are established.<sup>181</sup>

Seroprevalence investigations in developed regions have revealed the ubiquitous presence of anti-HEV antibodies in the analysed populations, although with significant differences between and within countries.<sup>11,172,182–186</sup> A significant concern is the high seroprevalence among US blood donors (18–21%)<sup>184,187</sup> compared with individuals professionally exposed to swine HEV, such as veterinarians (23–26%).<sup>184</sup>

Some studies have documented an increasing seroprevalence with age in both sexes, suggesting a continuous ongoing exposure to HEV.<sup>172,182,187,188</sup> In Japan, age-specific profiles of anti-HEV and anti-HAV antibodies suggest silent HEV infection in the last few decades, during which HAV infection rates declined.<sup>188</sup>

#### Zoonosis and host range

The possibility that HEV infection can be a zoonosis was raised when a virus, closely related to the HEV human strains, was isolated from pigs, initially in the USA<sup>189</sup> and, subsequently, worldwide.<sup>28,32–36,96,112,183,190–193</sup>

Experimental cross-species infections between swine and primates have shown that swine HEV could infect primates (surrogates for human infection), and the US-2 strain of human HEV (genotype 3) could infect specific pathogen-free pigs.<sup>194</sup> In contrast, US pigs could not support replication of human epidemic strains (genotypes 1 and 2).<sup>195</sup> Similarly, Indian pigs could be infected with swine HEV (genotype 4) but not with human HEV (genotype 1).<sup>112</sup>

These findings are supported by phylogenetic analysis data showing a high degree of nucleotide and amino-acid sequence homology between swine and human HEV isolates of genotypes 3 and 4 from the same geographical regions, suggesting that pigs may act as a reservoir for human infection.<sup>69,35,109,115,116,125</sup> On the contrary, swine HEV strains are highly divergent from the human strains of HEV classified within genotypes 1 and 2.<sup>69</sup>

Pigs are infected via the faecal–oral route<sup>196</sup> and develop a self-limiting subclinical infection<sup>189,197,198</sup> with transient viraemia (one to two weeks) but prolonged viral shedding (three to four weeks) in faeces. Current pig-raising practices perpetuate exposure of pigs to their waste, promoting viral transmission.<sup>196</sup>

Seroprevalence studies in pigs have shown the presence of HEV IgG in an unexpectedly high proportion of animals,<sup>36,199–201</sup> with peaks up to 85% in the UK,<sup>34</sup> 95–98% in India,<sup>127</sup> and 70–100% in Japan.<sup>109,202,203</sup> Although pigs are infected primarily at the early stage of production (1–3 months), HEV can still be detected by PCR at slaughter age, meaning that swine HEV can enter the food chain.<sup>28,36,98</sup> This has been shown in Japan, where infectious HEV was found in 2% of the pig liver packages ready for sale,<sup>39</sup> and more recently in the USA<sup>30</sup> and in The Netherlands.<sup>204</sup> HEV RNA was also detected in 3.1% of bile samples from swine in abattoirs in eastern China.<sup>28</sup>

HEV replicates in different visceral organs.<sup>192,205,206</sup> This explains the common foodborne transmission in Japan owing to the gastronomic habit of eating rarely, or poorly cooked, pig liver and intestines. Despite absence of evidence for HEV replication in muscles, HEV infection has been transmitted by consumption of meat from boar and deer as well.<sup>38,40,133</sup>

The finding of infectious HEV in pig-farm manure slurry samples<sup>193</sup> suggests that human exposure to swine waste may represent an alternative mode of transmission of zoonotic strains, particularly in regions where the water supply comes from wells, rivers, and streams, and where sewage treatment is not generally available.<sup>181</sup> In rural eastern China, where 9.6% of pig herds were found to be HEV RNA-positive by stool samples, a 74% higher risk of infection among people professionally engaged in swine farming was observed. Seroprevalence increased with the duration of occupational exposure to swine.<sup>28</sup> There was also a 29% higher risk of infection in people without occupational exposure to swine and residing in communities downstream of the Chinese swine farms, compared with those living in communities upstream.<sup>28</sup> Compared with control individuals, increased HEV IgG seroprevalence was also detected in people with occupational exposure to pigs in other countries, including Sweden and The Netherlands.<sup>183,207</sup>

Seroprevalence studies have shown that HEV natural infection is widespread in many species of wild and domestic mammals, including rats,<sup>208–212</sup> cattle,<sup>115,209</sup> goats,<sup>127</sup> wild mongooses,<sup>213</sup> monkeys,<sup>214</sup> dogs,<sup>215</sup> and pet cats,<sup>216</sup> with antibody positivity increasing with age in Japanese macaques and Japanese domestic pet cats.<sup>214,216</sup> Although these data show the prevalence of HEV circulation among animals, they have not established the possible role of these animals in transmitting HEV infection to humans. Of particular interest is the high HEV seroprevalence among wild rats,<sup>208–212</sup> as these rodents, ubiquitous worldwide, have the potential to be infected with swine and human HEV

strains. Rats may therefore be an important intermediate host between pigs and humans or, alternatively, a reservoir for both human and swine infection.

The majority of studies on animals other than swine have been more successful in detecting HEV antibodies than viral RNA. Although HEV RNA from genotypes 3 and 4 has been systematically found in pigs, boars, and deer, it has not yet been defined with certainty which genotypes circulate within other species. Two studies document HEV genotype 1 in horses<sup>217</sup> and pigs.<sup>218</sup> The finding of HEV genotype 1 in rats<sup>219</sup> has subsequently been found to be a laboratory error.<sup>220</sup> More studies are necessary to assess if HEV genotype 1 and 2 strains can induce sustainable infection in some animal species. In view of the large number of animal species that are potentially involved, further exploration of zoonotic transmission of HEV is warranted.<sup>23</sup>

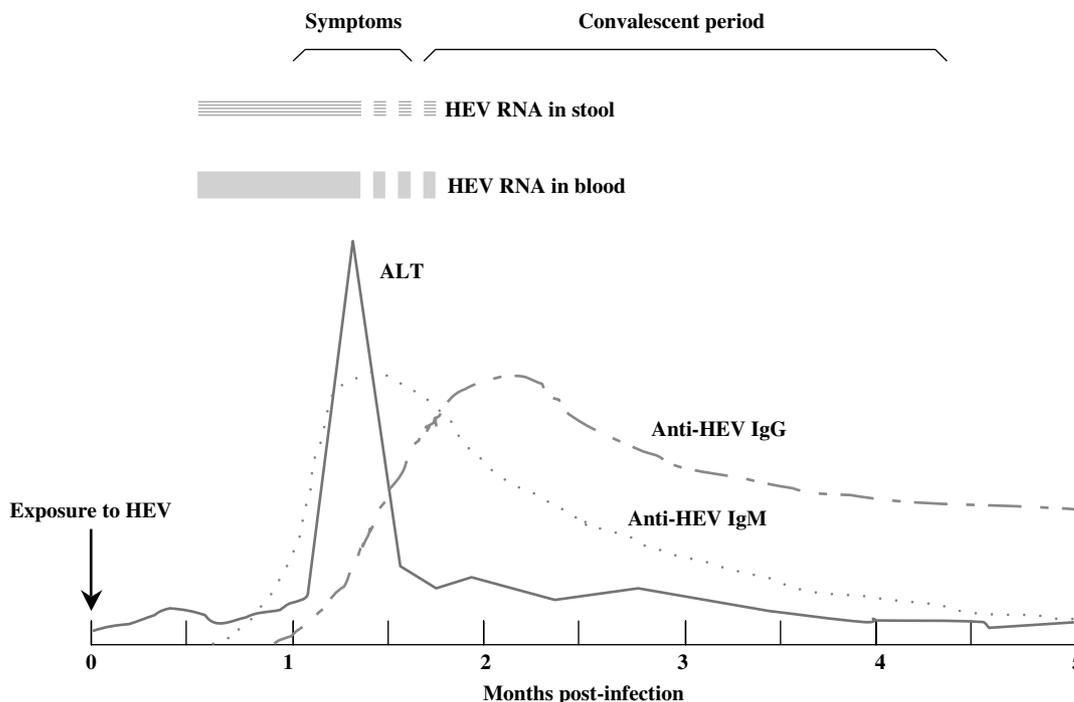
HEV strains were identified in poultry as well as mammals; phylogenetic analysis indicates that avian HEV is genetically related to, but distinct from, mammalian HEV strains<sup>221,222</sup> and does not represent a risk for cross-infection to humans.

### Pathogenesis, immune response, and time course of infection

It is thought that HEV infection initiates via cells lining the alimentary tract (primary site of virus replication) (Figure 4).<sup>223</sup> The virus then reaches the liver through the portal vein<sup>41</sup> and replicates in the cytoplasm of hepatocytes without causing direct cytolytic damage.<sup>223</sup> Several observations suggest that, in analogy with other hepatitis viruses, liver injury is largely immune-mediated:<sup>150,223,224</sup> first, viraemia precedes the onset of alanine transaminase elevation and liver histopathological changes,<sup>225–227</sup> secondly, experimental infection of nonhuman primates has shown how the liver damage coincides with the detection of serum anti-HEV antibodies and with a decreasing level of HEV antigens in the hepatocytes;<sup>150,225</sup> and finally, the lymphocytes infiltrating the liver have a cytotoxic/suppressor immunophenotype.<sup>150,225</sup>

HEV RNA is detectable in blood from as early as two weeks before<sup>173</sup> and for two to four weeks after the onset of symptoms.<sup>228–230</sup> HEV faecal excretion shows a similar temporal pattern.<sup>229</sup> Once liver function has normalised, HEV RNA is usually undetectable in blood and stool.<sup>229</sup> Viraemia and faecal shedding beyond the duration of biochemical hepatitis are uncommon,<sup>229–231</sup> suggesting that prolonged faecal shedding is not important in maintaining the environmental reservoir of HEV.<sup>229</sup>

The antibody responses are directed primarily against epitopes in the ORF2 and ORF3 proteins and are typically detectable at the onset of the disease, with IgM antibodies persisting for two to six months.<sup>223,228,232</sup> Anti-HEV IgG appears soon after IgM, and persists for a longer period of time.<sup>41,223,228,232,233</sup> However, the possibility of repeated infections being the cause of IgG persistence cannot be excluded.<sup>41</sup>



**Figure 4** Time course of HEV infection and specific humoral immune response. This figure shows the correlation of HEV RNA in stool and blood, sign of liver damage and development of anti-HEV antibody response with time. ALT, alanine aminotransferase; IgG, immunoglobulin G; IgM, immunoglobulin M.<sup>150,153,173</sup>

Overt disease in young adults is commonly the result of primary infection.<sup>234</sup> The importance of antibodies in protecting from clinical hepatitis E has been proven experimentally in primates, in which passive immunisation with anti-HEV antibodies was able to protect them against overt disease after challenge with virulent HEV.<sup>235</sup>

Limited data are available on anti-HEV cellular immune response. Evidence for anti-HEV T-cell response was provided by a study on patients with acute hepatitis E whose T-lymphocytes showed sensitisation to HEV peptides.<sup>236</sup> The same group was recently able to map CD4 T-cell epitopes in the ORF2 and ORF3 proteins of HEV using lymphocyte proliferation assays in patients with acute hepatitis E,<sup>237</sup> providing the basis for future studies on the immunopathogenesis of hepatitis E. No data are currently available regarding anti-HEV-specific CD8 T-cell responses or the role of cellular responses in the protection against viral infection.

Of great interest are the mechanisms determining the severity of disease during pregnancy, in which fulminant hepatitis is a common complication. HEV infection studied in pregnant and nonpregnant healthy women has shown that infection in pregnancy is associated with a shift in the Th cell type 1/Th cell type 2 balance toward Th cell type 2 response.<sup>238</sup> However, at this time it is difficult to link the clinical severity of the illness to this observation because the mechanism of liver injury in HEV infection has not yet been clarified.<sup>239</sup> A recent Indian study suggested that a subset of CD4-positive interferon- $\gamma$ -secreting cells, which do not belong to either the

helper Th cell type 1 or type 2 phenotype, might be involved in liver damage during acute HEV infection.<sup>240</sup>

## Clinical features

### Acute infection

The incubation period ranges from two to 10 weeks with an average of 40 days.<sup>43,163,228</sup> Hepatitis E is indistinguishable from other forms of viral hepatitis. Typical clinical features are one- to 10-day prodrome of malaise, fever, gastrointestinal symptoms (abdominal pain, anorexia, nausea, vomiting), followed by the onset of jaundice.<sup>41,150,155,161,241</sup> Once jaundice appears, prodromal symptoms subside. Clinical jaundice is not a constant feature and anicteric forms of hepatitis are well recognised.<sup>242</sup> Serum investigations reveal raised levels of bilirubin (predominantly conjugated) and alanine aminotransferase. The magnitude of the alanine aminotransferase elevation does not correlate with the severity of the liver injury, better expressed by the liver synthetic function, as determined by coagulation function estimation. Acute infection resolves in one to four weeks; however, some patients develop a more prolonged clinical illness with cholestasis (cholestatic hepatitis).<sup>150</sup> HEV infection is not known to progress to chronicity or cirrhosis<sup>2,243</sup> in immunocompetent patients.

### Complications

A small proportion of patients develop fulminant or subacute hepatic failure with high mortality as a result of

massive liver necrosis.<sup>150,244,245</sup> Fulminant hepatitis E has been described worldwide but it is particularly common in developing countries among pregnant women, mainly during the third trimester.<sup>164,239,246–249</sup> In this setting, HEV adversely affects both pregnant women and foetal outcome, with high mortality rate, increased frequency of abortions, preterm delivery, stillbirth, and neonatal death.<sup>23,99,148,150,239,250</sup>

In Japan, HEV genotype 4 appears to cause severe hepatitis more frequently than genotype 3,<sup>23,121,251</sup> possibly as a consequence of specific genomic mutations.<sup>119</sup> In Argentina, a country not endemic for hepatitis E, fulminant hepatitis has been recently diagnosed in three children infected with HEV genotype 3.<sup>99</sup>

Severe forms of hepatitis E have also been increasingly documented among patients, mainly men, with stable chronic liver disease of different aetiologies, including chronic hepatitis B and C, autoimmune hepatitis, alcoholic liver disease, cryptogenetic hepatitis, and Wilson's disease.<sup>165–171,252,253</sup>

Prolonged mild hepatitis with viral shedding has been described in immunocompromised patients during chemotherapy for T-cell lymphoma.<sup>143,254</sup> More recently, the evolution of HEV infection into chronic hepatitis E has been reported in solid organ transplant patients in France.<sup>255,256</sup> they not only persistently shed the virus in the presence of deranged alanine aminotransferase values, but also showed histopathological changes similar to those observed in chronic hepatitis C.

Uncommon HEV infection complications, described in anecdotal reports, include the Guillain-Barré syndrome,<sup>257</sup> acute transverse myelitis,<sup>258</sup> acute pancreatitis,<sup>259</sup> non-immune haemolytic anaemia,<sup>260</sup> lymphocytic destructive cholangitis,<sup>261</sup> and prolonged polyarthritis.<sup>262</sup>

### Asymptomatic infections

The number of asymptomatic infections far exceeds that of icteric hepatitis, as a large proportion of individuals who test positive for anti-HEV antibodies in highly endemic countries, such as India,<sup>150</sup> China,<sup>28,181</sup> and Egypt,<sup>179</sup> do not recall having suffered from jaundice. Similar data, based on seroprevalence, have been obtained from a variety of population profiles in developed countries: blood donors in Japan and the USA,<sup>140,184</sup> prisoners and drug users in Denmark,<sup>172</sup> and individuals living in the community in Spain.<sup>182</sup>

Direct evidence of ongoing subclinical HEV infection in the general population comes from a study conducted in Honshu (Japan) on 6700 asymptomatic blood donors with elevated aminotransferase levels during a three-year period: about 3% of the individuals with an aminotransferase level of  $\geq 201$  IU/l (normal value  $< 60$  IU/l) were HEV RNA-positive.<sup>140</sup> Based on the number of asymptomatic viraemic individuals and the incidence of clinical hepatitis E in Honshu, the authors estimate that less than 0.1% of HEV-infected cases exhibit clinical manifestation of the infec-

tion.<sup>140</sup> Asymptomatic viraemia has been detected in about 0.3% of individuals in rural eastern China.<sup>28</sup>

## Diagnosis

### Antibody detection

HEV antibody assays represent the routine diagnostic tool for acute hepatitis E cases. Test formats mostly consist of indirect enzyme-linked immunosorbent assays (EIA), with recombinant HEV proteins or peptides as detecting antigens. Currently available commercial assays are based mainly on HEV epidemic strains but EIA tests specific for genotypes 3 and 4 have also been developed.<sup>25,128,135</sup> The specificity and sensitivity of these tests have not been established with precision, limiting the reliability of laboratory results.<sup>263</sup>

One of the most widely available commercial antibody assays, the Genelabs-EIA, uses short recombinant proteins derived from the 3' termini of ORF2 (42 amino acids) and ORF3 (33 amino acids) from the Burmese (genotype 1) and Mexican (genotype 2) prototype sequences.<sup>263</sup> However, according to Zhou *et al.*,<sup>175</sup> a truncated form of the ORF2 protein, encompassing amino acids 112–607, contains the neutralisation epitopes (with amino acids 458–607 representing the major neutralisation site) and elicits the greatest and most long-lasting anti-HEV antibody response, being therefore suitable both for diagnostic and seroprevalence estimation purposes. In contrast, the amino acids 1–111 (N-terminus) and 607–660 (C-terminus) of the ORF2 protein and the ORF3 recombinant antigens elicit a weaker and transient antibody response, and are consequently of limited value in diagnosing acute HEV infection, and are not useful for seroprevalence studies.<sup>46,176</sup> Importantly, all genotypes share at least one major serologically crossreactive epitope, despite substantial genomic variability.<sup>62,174</sup>

Diagnosis of acute hepatitis E is made by detecting HEV-specific IgM in acute-phase sera or by detecting a rise in anti-HEV IgG titre between acute and convalescent serum samples. Cases of aberrant IgM and IgG serological profiles, including those with immunologically silent acute hepatitis E, have been documented.<sup>84,173</sup> Although atypical serological profiles from patients with proven HEV RNA viraemia may be the expression of a modified immune response, the insensitivity of diagnostic assays should be taken into account when interpreting these data.<sup>101,264,265</sup>

### Molecular detection

Reverse transcription-polymerase chain reaction (RT-PCR) assays represent the most commonly used molecular investigation for HEV genome detection. The usage of RT-PCR as a diagnostic tool has become feasible since the development of the real-time PCR platforms, closed systems that minimise the risk of contamination by the amplified target.

The majority of HEV RT-PCR assays used for diagnosis were developed as in-house assays by choosing different conserved HEV genomic regions as the target for amplifica-

tion.<sup>230,266–268</sup> Considering the wide genetic heterogeneity of HEV isolates, it is critical to design primers and probes that guarantee the development of highly sensitive and broadly reactive assays.<sup>268</sup>

RT-PCR is a useful complementary diagnostic tool for the diagnosis of acute HEV infection, as it can confirm cases of hepatitis E with atypical serological profiles. RT-PCR assays are also critically important for public health purposes when used for detecting HEV-contaminated environmental samples.

## Prevention and control of the infection

### Active immunisation

The observation that passive immune prophylaxis with convalescent serum samples prevented hepatitis E in primates has indicated that vaccination against HEV based on humoral immunity is feasible.<sup>55,235,269</sup> This has prompted HEV immunisation studies based mainly on recombinant proteins, because the unavailability of an efficient cell culture system for HEV replication<sup>270</sup> has precluded the development of vaccines based on inactivated or attenuated whole-virus particles. However, other approaches, such as DNA-based vaccines,<sup>271,272</sup> able to induce both cellular and antibody response, are also under evaluation.

The ORF2 protein has been considered the best candidate for HEV vaccine because it contains the neutralisation epitope located between amino acids 458 and 607<sup>56</sup> and is crossreactive with all mammalian HEV.<sup>56,273</sup> Animal studies have shown that ORF2 recombinant proteins<sup>274–278</sup> elicit

neutralising antibodies and mediate protective immunity in vaccinated primates.<sup>277,279</sup>

One such vaccine with a 56 kDa protein encompassing amino acids 112–607<sup>55,280</sup> was recently evaluated in young adults in Nepal in a phase 2, randomised, double-blind, placebo-controlled trial.<sup>281</sup> The study had encouraging results establishing that three doses of hepatitis E vaccine were 95.5% effective in protecting against clinical hepatitis E after a median of 804 days. The primary endpoint of this study was the prevention of clinically overt HEV infection, but the ability of the vaccine to prevent asymptomatic infection and asymptomatic virus shedding was not investigated.<sup>174</sup> Asymptomatic HEV shedding in vaccine recipients, shown previously in primates,<sup>280</sup> may be relevant in maintaining the environmental reservoir of HEV for human infection.<sup>174</sup> Another aspect that this trial could not clarify is the duration of the induced immunity. Based on currently available data, this vaccine may be useful for travellers to highly endemic areas and for susceptible pregnant women,<sup>174</sup> particularly during outbreaks. However, its use in children and adolescents in hepatitis E endemic countries,<sup>174</sup> or in individuals with chronic liver disease, requires further study assessing the duration of its protective efficacy.

### Protection of the environment and control of the outbreaks

The most important measure to prevent HEV infection is the protection of water supply from faecal contamination.

**Table 1** Clinical–epidemiological characteristics of HEV infection

<i>HEV Genotypes</i>	<i>Geographical distribution</i>	<i>Hosts</i>	<i>Mode of transmission</i>	<i>Epidemic versus sporadic forms</i>	<i>Clinical attack rate</i>	<i>Disease severity</i>
1	Asia Africa	Humans ? Animals	Contamination of water supply	Sporadic and epidemic forms	Highest in young adults	Highest in pregnant women and individuals with chronic liver disease
2	Central America, Africa					
3	Asia, America, Europe, Oceania	Humans and a variety of animals, particularly swine	Food (Japan)	Sporadic and small outbreaks	Highest in older males	Highest in individuals with chronic liver disease
4	Asia		Food (Japan), <i>Environmental contamination by swine waste</i>			

Abbreviation: HEV, hepatitis E virus. Zoonotic strains (HEV genotype 3 and 4) cause infection both in developed and in developing countries (in italics), such as China.

Chlorination and filtration systems are generally inadequate if the source water is heavily contaminated.<sup>223</sup> Travellers to highly endemic regions should strictly consume only bottled or boiled water.

During outbreaks, it is critical to provide clean water to all pregnant women. The isolation of individuals affected by acute hepatitis E is not justified because person-to-person transmission is uncommon. Infected people should refrain from food handling and food preparation.<sup>223</sup>

## Conclusion

HEV infection has complex, and not yet completely clarified, clinical-epidemiological characteristics, which are summarised in Table 1. Two forms of infection have been identified: hepatitis E caused by epidemic strains, affecting mainly young adults and particularly pregnant women, and hepatitis E caused by zoonotic strains, which mostly affect older males.<sup>282</sup> These differences in sex- and age-specific attack rates are puzzling because the route of transmission in developed countries, apart from Japan, has not yet been identified.

More extensive epidemiological studies are needed, not only to assess the HEV seroprevalence in humans, but also in several animal species. For this purpose, the development of broadly reactive reliable antibody assays, which include immunodominant neutralisation antigens, is critical. Equally important is to establish which HEV genotypes circulate among the different animal species, and their role in human infections.

A high level of suspicion is needed in developed countries where the increasing number of recognised cases of hepatitis E<sup>16,18,100-102</sup> suggests significant underdiagnosis.<sup>100</sup> Awareness of HEV infection should exist in immunocompromised patients with signs of liver damage in view of the recent finding that hepatitis E can evolve to a chronic infection.<sup>255,256</sup>

The results of a HEV vaccination phase 2 trial have provided encouraging preliminary data for the prevention of hepatitis E.<sup>281</sup> Data are now awaited regarding the duration of the immune response induced by the vaccine before routine immunisation of children can be promoted in epidemic countries. It will also be important to establish the vaccine efficacy among older adults and the elderly, who are targets of overt HEV infection and in whom most cases of chronic liver disease, a risk factor for severe hepatitis E, concentrate.

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### Competing interest

The authors state no competing interests.

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